

AJMHR

Asian Journal of Medical and Health Research Journal home page: www.ajmhr.com

Antimicrobial and Larvicidal Activity Of Bauhinia Recemosa

Sutha P^{*1}, Sangameswaran B². 1.SSM College of Pharmacy, Bhavani, Erode, Tamilnadu, India

ABSTRACT

Bauhinia racemosa was used traditionally to take care of analgesic, anti-pyretic, anti inflammatory, antioxidant activity, anti-spasmodic, and antimicrobial activity properties. In this context, I decided to evaluate the antimicrobial and larvicidal property of Bauhinia racemosa. The aim of the study was to investigate the antimicrobial and larvicidal activities of plant extracts (aqueous, ethanol) from *Bauhinia racemosa* against ten microorganisms comprising of eight bacteria (Streptomyces fulvissimus, Klebsiella pneumonia, Shigella flexneri ,Escherichiae coli, Bacillus subtilis, Streptococcus pyogenes, Pseudomonus aeuruginosa, Proteus mirabilis) and two fungi (Aspergillus niger, Candida albicans) using well diffusion method and larvicidal activity were tested on the larvae of the dengue-vector, Aedes aegypti. Phytochemical screening of the extracts was conducted to determine the active toxic compounds. Various concentrations (20, 40, 60,80 mg/ml) of the plant extracts were tested against fourth instar larvae of A. aegypti. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, glycosides and steroids. These compounds are known to possess insecticidal and larvicidal properties causing the mortality of insects and other pests. Alcohol and aqueous extracts showed significant larvicidal activity against A. aegypti mosquito larvae. Insecticidal susceptibility tests were carried out using WHO standard method and the mortality was observed after 24 and 48 hours exposure. The tested extracts showed moderate to good larvicidal activities. However, the maximum larval mortality was detected in ethanol extract. Ethanol extract showed larvicidal activity against A. aegypti with LC₅₀ value of 76mg/ml & 64mg/ml at 24 & 48 hours respectively. Also exhibited the LC₉₀ value of 117mg/ml & 100mg/ml at 24 & 48 hours respectively. Similarly, Aqueous extracts of Bauhinia recemosa showed larvicidal activity against A. aegypti with LC₅₀ value of 86mg/ml & 70mg/ml at 24 &48 hours respectively. Also exhibited the LC₉₀ value of 139 &125 mg/ml at 24 & 48 hours respectively. The results of the present study revealed that ethanol extract possessed high larvicidal activity when compared to aqueous with LC₅₀ and LC₉₀ values.

Keywords: Larvicidal activity(*Ades aegpyti*), Antimicrobial activity, Aqueous & Ethanol extracts, *Bauhinia recemosa*

Received 13 March 2019, Accepted 22 March 2019

Please cite this article as: Sutha P *et al.*, *Antimicrobial and Larvicidal Activity Of Bauhinia Recemosa*. Asian Journal of Medical and Health Research 2019.

INTRODUCTION

Mosquitoes serve as vector for various tropical and subtropical diseases which cause destructive effects to human. The most common diseases associated with mosquitoes are dengue fever, chikungunya, yellow fever and the worst, dengue hemorrhagic fever where Aedes aegypti is one of the mosquito species responsible for the transmission of these vector borne diseases. World Health Organization (WHO) stated that about 2/5 of the global human population are currently threaten of dengue and the best way to control the transmission of dengue virus is fight the mosquitoes that cause the disease. ¹

Dengue is one of the most significant viral diseases transmitted by Aedes aegypti because it afflicts humans worldwide whose symptoms ranging from mild fever to a severe and potentially life threatening hemorrhagic disease. Aedes aegypti is of supreme concern because of its wide distribution and close association with humans.²

In recent years, use of many of the former synthetic insecticides in mosquito control programme has been limited. It is due to lack of novel insecticides, high cost of synthetic insecticides, concern for environmental sustainability, harmful effect on human health.³ The control of mosquito at the immature stage is necessary and efficient in integrated mosquito management because during the immature stages, mosquitoes are immobile. The use of natural products for the control of insect pests offers an economically viable and eco-friendly approach.⁴

Microorganisms have developed resistance to many antibiotics and this has created vast clinical inconvenience in the treatment of infectious diseases. The increase in microorganisms resistance to antibiotics, the use of antimicrobial drugs forced scientists to search for new antimicrobial substances from various sources including medicinal plants.⁵

Medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs. A wide range of medicinal parts are used to get different rasayanas which possess different medicinal properties against different microbes.⁶ Some amount of pharmacological work has been carried out on *Bauhinia racemosa*. It is evident that the Indian species *Bauhinia racemosa* was used traditionally to take care of analgesic, anti-pyretic, anti inflammatory, antioxidant activity, anti-spasmodic properties.⁷ But no study is found regarding the investigation of larvicidal activity of *Bauhinia racemosa*. Therefore, in this present study, I tried to carry out preliminary phytochemical screening, antimicrobial activities and pharmacological investigation of aerial parts of *Bauhinia racemosa* for larvicidal activity.

MATERIALS AND METHOD

Plant materials:

The aerial parts of the *Bauhinia racemosa* was collected near Bargur hills, Erode district, Tamilnadu, India. The plant material was authenticated by Dr.G.V.S. MURTHY, Scientist 'G' and Head of office , **Botanical Survey Of India , Coimbatore, Tamilnadu, India** and a voucher specimen BSIS/RC/5/23/2016/Tech,/2058 was deposited at the museum, SSM college of Pharmacy, Erode (638312) Tamil nadu, India.

Preparation of extracts:

The shade dried powder material of whole plant of *Bauhinia racemosa* was extracted with solvents (ethanol and water) by maceration method for 3 days and then filtered through Whatman filter paper (3 mm). Then extract was concentrated to a distillation apparatus.^{8,9}

Anti-microbial activity

Twelve microorganisms comprising of eight bacteria (*Streptomyces fulvissimus*, *Klebsiella pneumonia ,Shigella flexneri*, *Escherichiae coli*, *Bacillus subtitis*, *Streptococcus pyogenes*, *Pseudomonus aeruginosa*, *Proteus mirabilis* and two fungi (*Aspergilus niger*, *Candida albicans*) were obtained from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh. The typed cultures of bacteria and fungi were sub-cultured on nutrient agar(NA) and Sabourts dextrose agar (SDA) slants respectively and stored at 4 °C until required for study. The anti-microbial assay was performed by agar well diffusion method. 0.6ml of standardized bacterial stock suspension was thoroughly mixed with 60ml of sterile nutrient agar. 20ml of the inoculated nutrient agar were distributed into sterile petri dishes. The plates were allowed to dry for at least 15 minutes. A sterile cork borer No.4 was used to make wells of 6 mm diameter in each plate. A total of 0.2ml of plant extracts were poured into the wells with concentrations as $1000\mu g/ml$, $500\mu g/ml$, $250\mu g/ml$, and incubated overnight at 37 °C. Each test was repeated triplicate.^{10,11,12, 13} The obtained results were compared with the standard (Oxytetracycline). The same procedure was adopted for fungal species with Ketoconazole was the standard.¹⁴

Test insects

A. aegypti, larvae was obtained from National Centre for Disease Control (NCDC) Coonoor, Tamil Nadu. Larvae were fed a diet of Brewer's yeast and powdered dog biscuits in the ratio of 3:1, kept at $27 \pm 2^{\circ}$ C and 75% - 85% relative humidity (RH), with a photoperiod of 14:10 LD for the larval growth. Fourth instars larva were used for larval bioassay.^{1,15}

Identification of Mosquito Larvae:

Larvae of a mosquito can be identified from any other aquatic insects since it has a combination of two characters, they have no legs and the thorax is wider than the head or abdomen. The

three divisions of the body part mosquito larvae are head, thorax and abdomen. The structure of three body regions serves as the basis for identifying the mosquito larvae. The mosquito larva was identified using a compound microscope. A small amount of water with a mosquito larvae was drop in a slide to be able to view the specimen in the compound microscope. The target mosquito larva in this study was the fourth instars larva of dengue carrying mosquito *Aedes aegypti*. *Aedes aegypti* larvae can be distinguished from any other mosquito larvae since it normally has a single hair, a three branch hair tufts on each side of the air tube. When the hair tuft has two or more branches all branches arise from the same socket. Other species have two or more hairs, branches and hair tufts on each side of the air tube or siphon. Identified *Aedes aegypti* mosquito larvae were separated from the other mosquito species and were placed in a water- filled plastic molder.²

Experimental design

The plant extracts were dissolved in 10µl of DMSO for its solubility in water. The larvicidal activity was assessed by the procedure of WHO with some modification. Twenty healthy larvae were released into each 250 ml glass beaker containing 200 ml of test concentration. Mortality was observed for 24 and 48 hours after treatment. The larvae were considered dead when they showed no sign of movement when they were probed using a needle. A total of three trials with three replicates per trial for each concentration were carried out. Controls were run simultaneously. Distilled water served as control. One way ANOVA was performed to determine the difference in larval mortality between concentrations. ^{16,17} The larval percentage mortality was calculated by using following formula

Percentage of mortility = $\frac{\text{Number of dead larvae}}{\text{Number of larvae introduced}} \times 100$

Statistical Analysis: The statistical tools that were used in this study are the following: the Arithmetic Mean to get the average number of dead of mosquito larvae, Analysis of Variance (ANOVA), to determine the significant difference on the mortality of mosquito larvae between the control and the experimental groups, and Probit Analysis to calculate LC_{50} and LC_{90} values to determine Lethal concentrations of the plant extracts on *Aedes aegypti* mosquito larvae after 24 and 48 hours of treatment. ^{18,19,20}

RESULTS AND DISCUSSION

The ethanol and aqueous extracts showed more active constituents were utilized for the antimicrobial and larvicidal studies. Eight bacteria and four fungi were used for antimicrobial screening. Various concentrations of aqueous and ethanol extracts (1000µg/ml, 500µg/ml, 250µg/ml) were used for testing the antimicrobial activities. The results were shown in the

table 1-3 which indicated that, the extracts showed inhibition of growth against tested microorganisms. Successful prediction of extracted compounds from plant materials largely dependent on the type of solvent used in the extraction procedure. The traditional practitioners make use of water as a primer solvent, but on the first observation ethanol was a better solvent for extracting antimicrobial substance, Ethanol extract of *Bauhinia racemosa* reported to be more effective against fungal and bacterial species showing highest inhibition against fungi such as 12mm against C.*albicans*, 16mm against *A.niger* and bacteria 18mm against *Pseudomonas aeruginosa*, 15mm against *Streptococcus pyogenes*. Similarly, aqueous extract of *Bauhinia racemosa* showed more activity 10mm against *Pseudomonas aeruginosa*, 9mm against *Streptococcus pyogenes*. Also tested against fungal species, it showed the inhibition of 8mm in *A.niger* and 6mm against *C.albicans*. The overall comparative studies showed that the ethanol extract of *Bauhinia racemosa* showed highest degree of antibacterial and antifungal activity than aqueous extract.

Test organism	Zone	of inhi	emosa	Oxytetracycline			
	Aqueous extract(µg/ml)			Ethano (µg/ml)	ol extra)	(mg/ml)	
	1000	500	250	1000	500	250	1
E.coli	12	09	07	06	04	03	21
K.pneumoniae	07	03	02	05	03	02	22
P.mirabilis	13	10	08	07	04	03	20
S.flexneri	11	08	06	06	04	02	21
S.pyogenes	15	12	07	09	07	03	24
S.fulvissimus	14	10	07	08	06	04	22
B.subtilis	12	09	06	05	03	00	23
P.aeruginosa	18	13	09	10	06	02	22

Table 1.	In vitro	Anti bacterial	assav of	Aqueous and	Ethanol extracts of	Opuntia stricta.
I UNIC II		i inti succei iu	ubbuy or	riqueous una	Emanor extracts of	opunna snicia.

mg – milligram, µg-microgram, ml-millilitre

Test organism	Zone	of inhi	Ketoconazole				
	Aqueous extract(µg/ml)			Ethan (µg/m	ol exti l)	(<i>mg/ml</i>)	
	1000	500	250	1000	500	250	1
C.albicans	12	08	03	06	04	02	19
A.niger	16	10	06	08	05	03	21

mg - milligram, μg -microgram, ml-millilitre

Plant	Conc	Percentage mortality											
Name	(mg /	Ethanol extract						Aqueous extract					
	ml)	24h	LC50	LC90	48h	LC50	LC90	24h	LC50	LC90	48h	LC50	LC90
Bauhinia	Control	0	76	117	0	64	100	0	86	139	0	70	125
racemosa	20	10.2 ± 0.4			20.1±0.3			5			10		
	40	20.6 ± 0.8			30.4 ± 0.8			15			25		
	60	35.4±1.3			45.2 ± 0.4			30			40		
	80	55.8 ± 1.0			70.8 ± 0.9			45			60		
	100	75.9 ± 0.9			90.2±1.2			60			75		

Table 3 : Larvicidal activity of B. racemosa

CONCLUSION

The antibacterial and antifungal properties of two extracts were identified. The results indicated that, the extracts obtained from the plants showed inhibition of growth against tested microorganisms. The overall comparative studies showed that the ethanol extract of Bauhinia racemosa showed highest degree of antibacterial and antifungal activity than aqueous extract. The plant's larvicidal activity is supported by the presence of phytochemicals such as alkaloids, saponins, flavonoids, steroids and tannins which showed combination effects in terms of larvicidal action to mosquito larvae. Phytochemicals derived from plant sources can act as larvicide, insect growth regulators, & repellent. Plant could be an alternative source for mosquito larvicide because they constitute a potential source of bioactive chemicals and generally free from harmful effects. Use of these botanical derivatives in mosquito control instead of synthetic insecticides could reduce the cost and environmental pollution. The present study shows the ethanol extracts of Bauhinia racemosa shows 100% mortality after 48hrs of incubation. Moreover behavioral changes were observed in the movement of the larvae. These effects may be due to the presence of neurotoxin compounds in plant extracts. No behavioral changes were obtained in control group. Further analysis is required to isolate the active principles and its mode of action in inhibiting the developmental stages in Aedes aegypti. The phytochemicals of Bauhinia racemosa extracts can be well utilized for preparing biocides or insecticidal formulation. The overall comparative studies showed that the ethanol extract of Bauhinia racemosa showed highest degree of larvicidal activity than aqueous extract.

REFERENCE:

- Shivakumar M S, Srinivasan R and Natarajan D. Larvicidal potential of some Indian medicinal plant extracts against *Aedes aegypti*. 2013. *Asian J pharm clin res*, Vol 6(3) , 77-80
- Pedro M Gutierrez, Aubrey N Antepuesto, Bryle Adrian L Eugenio. 2014. Larvicidal Activity of Selected Plant Extracts against the Dengue vector *Aedes aegypti* Mosquito. *Int. Res. J. Biological Sci*.Vol. 3(4), 23-32.

- Anupam Ghosh, Nandita ChowdhuryGoutam Chandra. 2012. Plant extracts as potential mosquito larvicides. Indian J Med Res. 135(5), 581–598.
- Samuel Tennyson K John Ravindran S Arivoli. Screening of twenty five plant extracts for larvicidal activity against Culex quinquefasciatus Say (Diptera: Culicidae). 2012. *Asian Pacific J Tropical Biomed*. 1130-1134
- Chandrakant D, Shendkar, Pranav S, Chandrachood, Sangita M Lavate, Bipinraj N Kunchiraman. Comparative evaluation of *Achyranthes aspera* Linn. parts by antibacterial activity. J Pharm Res. 2012; 5(1):102-103.
- Mahesh B and Satish S. 2008. Anti microbial activity of some important medicinal plant against plant and human pathogens. *World J. of agriculture sci.* Vol 4(S), 839-843.
- 7. GoutamGhosh, Pritipadmapanda, Debajyotidas, priyankadash.2015. Therapeutic potential of *Bauhinia racemosa*. *A mini rev Int. J. Pharm, sci.* Vol 32 (2)(28) 169-179.
- 8. Zineb Dahchar, Fatiha Bendali-Saoudi, Noureddine Soltani. Larvicidal activity of some plant extracts against two mosquito species *Culex pipiens* and *Culiseta longiareolata*
- Stephen Olaribigbe., Majekodunmi., 2015. "Review of extraction of medicinal plants for pharmaceutical research" *Merit Res. J. of Medicine and Medical Sci.* vol 3(11), 521-527.
- Memnune Sengul., Hilal Yildiz., Neva Gungor., Bulent Cetin., Zeynep Eser., Sezai Ercisli., 2009. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pak. J. Pharm. Sci 22(1), 102-106.
- Saravanakumar, A., Venkateshwaran, K., Vanitha, J., Ganesh, M., Vasudevan, M., and Sivakumar, T., 2009. Evaluation of antibacterial activity, phenol and flavonoid Contents of *Thespesia populnea* flower extracts. Pak. J.Pharm. Sci. 22(3), 282-286.
- Adegoke., Anthony, A., Adebayo-tayo., Bukola, C., 2009. Antibacterial activity and phytochemical analysis of leaf extracts of *Lasienthera africanum*. African Journal of Biotechnology 8 (1), 077-080.
- Anupam Ghosh., Bidus Kanti Das., Soroj Kumar Chatterjee., Goutam Chandra., 2008. Antibacterial potentiality and phytochemical analysis of mature leaves of *Polyalthia longifolia (Magnoliales: Annonaceae)*. The South Pacific Journal of Natural Science 26, 68-72.
- 14. Somaie Shafiei., Ashrf Kariminik., Zahra Hasanabdi, 2013. "Antimicrobial activity of menthol extracts of *opuntia stricta F*" *Int. J. Applied and basic sci.* Vol 7(12), 907-910.
- 15. Anitha Rajasekaran S, Geethapriya Duraikannan. 2012.Larvicidal activity of plant extracts on *Aedes Aegypti* L. *Asian pacific J. of tropical bio medicines*. 1578–1582.

- 16. Raveen R, Kamakshi K T, Deepa M, Arivoli S and Samuel Tennyson. 2014. Larvicidal activity of *Nerium oleander* L. (*Apocynaceae*) flower extracts against *Culex quinquefasciatus* Say (*Diptera Culicidae*). Int J. of Mosquito Res. Vol 1(1), 38-42.
- Sakthivadivel M, Daniel T. 2008. "Evaluation of certain insecticidal plants for the control of vector mosquitoes viz, Culex quinquefasciatus, Anopheles stephensi and Aedes aegypti" Applied Entomology and Zoology. Vol 43(1), 57-63.
- Abdelouaheb Alouani, Nassima rehimi, Noureddine soltani. 2009. Larvicidal activity of neem tree extract (*azadirachtin*) against mosquito larvae in the republic of Algeria. Vol 2(1), 15 22.
- Ram Kumar and Jiang-Shiou Hwang, 2005. "Larvicidal Efficiency of Aquatic Predators: A Perspective for Mosquito Biocontrol" *Zoological Studies*. Vol 45(4), 447-466.
- 20. Radhika W, Naim W and Sarita K, 2011. "Larvicidal potential of commercially available pine (*Pinus longifolia*) and cinnamon (*Cinnamomum zeylanicum*) oils against dengue fever mosquito Aedes aegypti L. (Diptera Culicidae)" Fi Acta Entomologica Sinka. Vol 54(7), 793 -799.

AJMHR is

- Peer reviewed
- Monthly
- **Rapid publication**
- Submit your next manuscript at info@ajmhr.com

